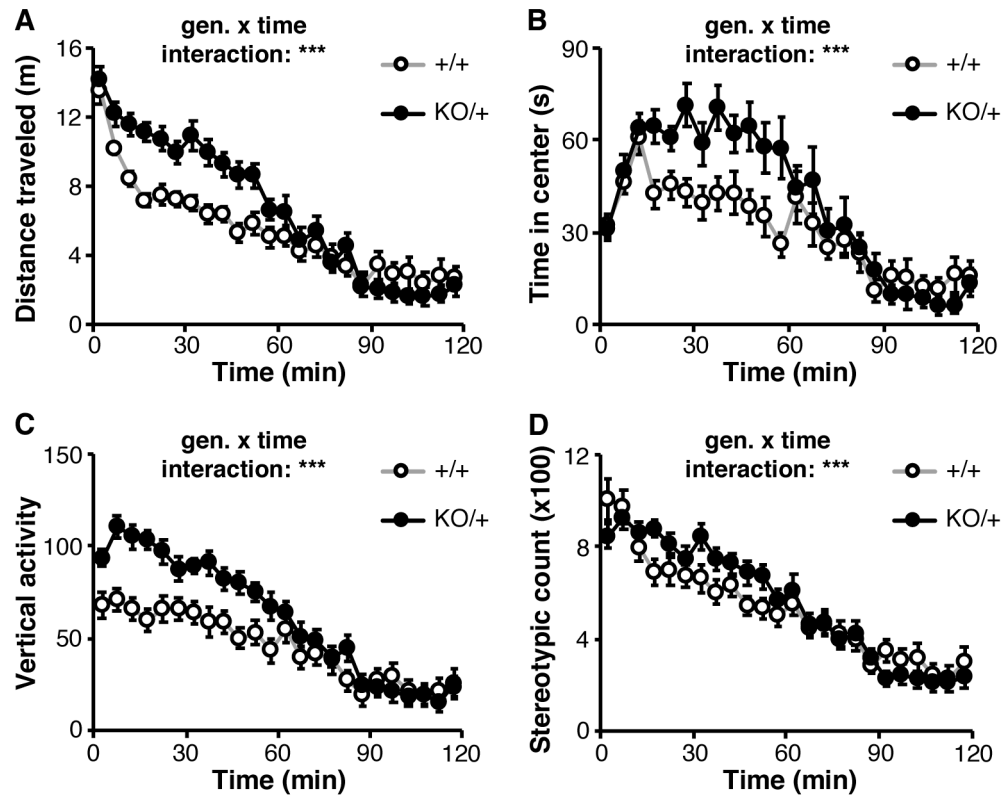
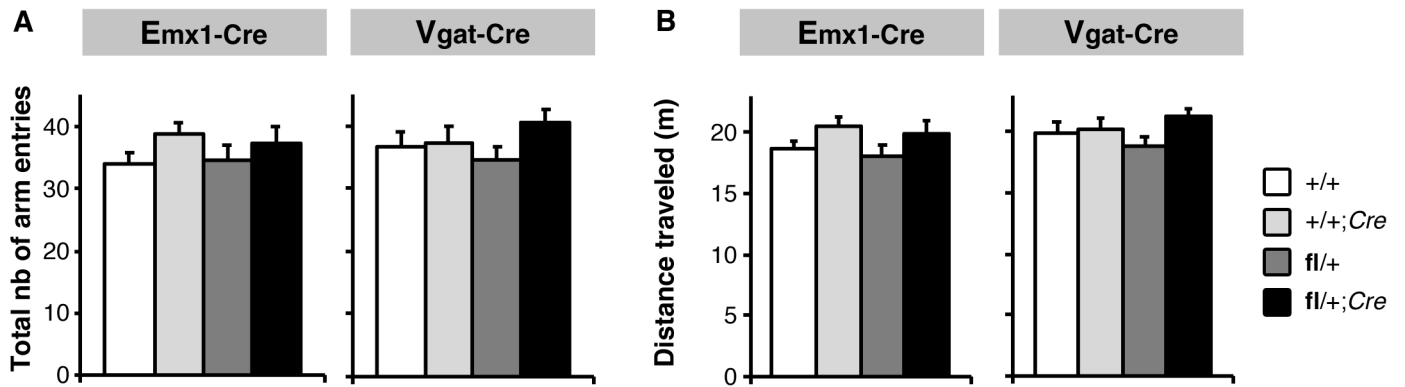


**Figure S1. General health and motor function in *Scn2a* KO mice.** At 11-weeks-old, body weight (**A**) and temperature (**B**) were not significantly different between *Scn2a*<sup>KO/+</sup> mice and their WT littermates. In the hot plate test (**C**) the latency to sensory response was comparable between the two groups. The grip strength (**D**) and the performance in the wire hang test (**E**) in *Scn2a*<sup>KO/+</sup> mice was comparable to that of their littermates. However, *Scn2a*<sup>KO/+</sup> mice performed significantly worse in the rotarod task (**F**) as their latency to fall off the rod was significantly shorter than their WT counterparts (genotype-trial interaction:  $F_{5,95} = 1.095$ , *NS*; trial effect:  $F_{5,95} = 16.220$ ,  $p < 0.001$ ; genotype effect:  $F_{1,19} = 32.440$ ,  $p < 0.001$ ). WT: N = 20, KO: N = 20. Values are expressed as mean  $\pm$  standard error of the mean. Statistical significance was assessed using one-way ANOVA (A-E) or two-way repeated measures ANOVA (F) with significance set at (\*)  $p < 0.05$ , (\*\*)  $p < 0.01$  and (\*\*\*)  $p < 0.001$ .



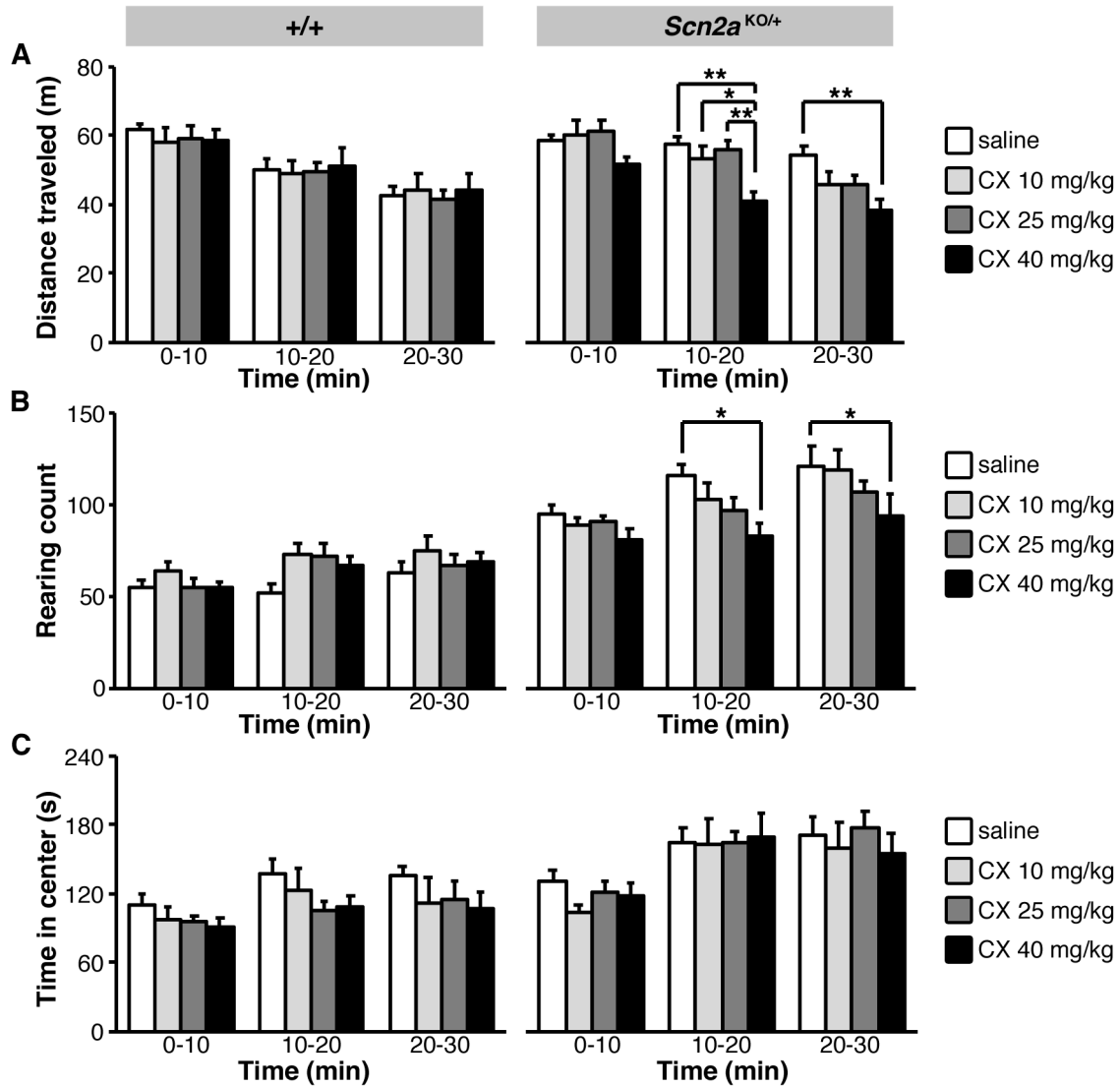
**Figure S2. *Scn2a*<sup>KO/+</sup> mice show hyperactivity and low anxiety during the first 60 min of exploration in a new environment.** (A) When *Scn2a*<sup>KO/+</sup> mice were left to explore freely an open field over a 2 hours period, they traveled a significantly longer distance during the initial 60 minutes (genotype-time interaction:  $F_{23,437} = 5.832$ ,  $p < 0.001$ ; post-hoc time points 3 to 11:  $p < 0.01$ ). They also spent significantly more time at the center of the open field (B, genotype-time interaction:  $F_{23,437} = 3.725$ ,  $p < 0.001$ ; post-hoc time points 4, 6, 8, 10-12:  $p < 0.05$ ) and reared significantly more (C, genotype-time interaction:  $F_{23,437} = 5.698$ ,  $p < 0.001$ ; post-hoc time points 1 to 12:  $p < 0.05$ ) than their WT littermates. The total number of stereotypic counts (D) was however overall not significantly different between the groups (genotype-time interaction:  $F_{23,437} = 3.043$ ,  $p < 0.001$ ; post-hoc time points 4 and 7:  $p < 0.05$ ). WT: N = 20, KO: N = 20. Values are expressed as mean  $\pm$  standard error of the mean. Statistical significance was assessed using two-way repeated measures ANOVA with significance set at  $p < 0.05$ .

# ELEVATED PLUS MAZE



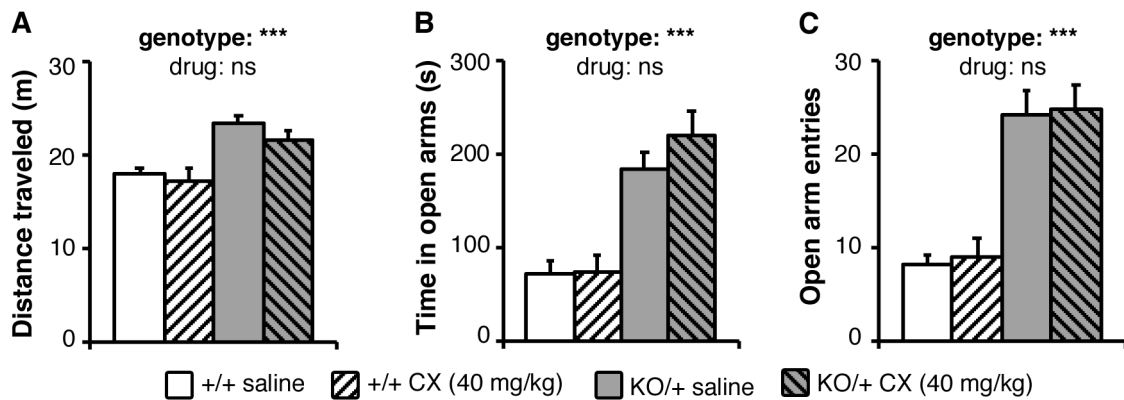
**Figure S3. Exploratory activity is not significantly affected in the elevated plus maze task in *Emx1*- and *Vgat*-Cre conditional *Scn2a* knockout mice.** (A) The total number of arm entries in the elevated plus maze task was not significantly affected in mice carrying a heterozygous deletion of *Scn2a* in dorsal-telencephalic excitatory neurons (*Emx1*-Cre) nor in mice with a heterozygous deletion of *Scn2a* in inhibitory neurons (*Vgat*-Cre) (*Emx1*-Cre:  $F_{3,53} = 1.036$ , NS; *Vgat*-Cre:  $F_{3,46} = 1.331$ , NS). (B) No significant differences were observed in the total traveled distance (*Emx1*-Cre:  $F_{3,53} = 1.595$  NS; *Vgat*-Cre:  $F_{3,46} = 1.554$ , NS). Values are expressed as mean  $\pm$  standard error of the mean. Statistical significance was assessed using one-way ANOVA with significance set at  $p < 0.05$ .

## OPEN FIELD

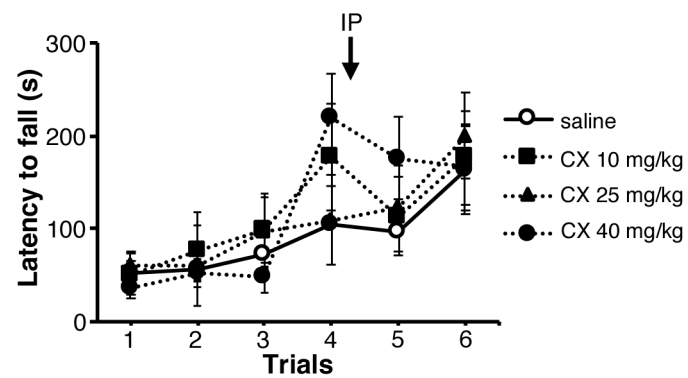


**Figure S4. Treatment by AMPA receptor modulator CX516 shows a maximal effect at 40 mg/kg for spontaneous exploratory behavior assessment.** CX516, a specific AMPA receptor positive allosteric modulator, was intraperitoneally administered 10 minutes before submitting WT (+/+) and *Scn2a*<sup>KO/+</sup> mice to the open field task. **(A)** The distance traveled by WT mice decreased over time without a significant effect of CX516 injections at the 3 doses tested (time-drug dose interaction:  $F_{6,80} = 0.562$ , NS; time effect:  $F_{2,80} = 173.290$ ,  $p < 0.001$ ; drug dose effect:  $F_{3,40} = 0.044$ , NS). In *Scn2a*<sup>KO/+</sup> mice there a CX516 injections led to a significant decrease in traveled distance in the second and third blocks of 10 minutes, with the strongest effect at 40 mg/kg (time-drug dose interaction:  $F_{6,80} = 2.426$ ,  $p < 0.05$ ). **(B)** The rearing count in the WT group showed a slight but significant increase over time without a significant effect of CX516 (time-drug dose interaction:  $F_{6,80} = 1.075$ , NS; time effect:  $F_{2,80} = 6.859$ ,  $p < 0.01$ ; drug dose effect:  $F_{3,40} = 1.490$ , NS). *Scn2a*<sup>KO/+</sup> mice reared significantly more during the second and third blocks of 10 minutes and CX516 injections suppressed this behavior in a dose dependent manner with a maximum effect at 40 mg/kg (time-drug dose interaction:  $F_{6,80} = 1.765$ , NS; time effect:  $F_{2,80} = 15.410$ ,  $p < 0.001$ ; drug dose effect:  $F_{3,40} = 3.866$ ,  $p < 0.05$ ). **(C)** Over the course of the test, WT mice gradually spent more time in the center and CX516 did not affect significantly this behavior (time-drug dose interaction:  $F_{6,80} = 0.408$ , NS; time effect:  $F_{2,80} = 6.626$ ,  $p < 0.01$ ; drug dose effect:  $F_{3,40} = 1.074$ , NS). In a similar way, the time spent in the center in the *Scn2a*<sup>KO/+</sup> group increased over time and was not affected by CX516 at the three doses tested (time-drug dose interaction:  $F_{6,80} = 0.487$ , NS; time effect:  $F_{2,80} = 23.330$ ,  $p < 0.001$ ; drug dose effect:  $F_{3,40} = 0.271$ , NS). The test was conducted using  $N = 11$  mice per genotype and per condition (saline, CX516 at 10, 25 or 40 mg/kg). Values are expressed as mean  $\pm$  standard error of the mean. Statistical significance was assessed using two-way repeated measures ANOVA for treatment and time separately in each group of genotype, followed by Tukey's post-hoc test with significance set at (\*)  $p < 0.05$  and (\*\*)  $p < 0.01$ .

# ELEVATED PLUS MAZE

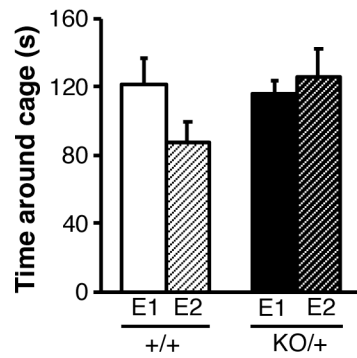


**Figure S5. Treatment with the AMPA receptor modulator CX516 did not affect the decrease in anxiety-related behavior of *Scn2a*<sup>KO/+</sup> in the elevated-plus maze.** (A) Saline injected *Scn2a*<sup>KO/+</sup> mice (KO/+) traveled longer distances in the elevated-plus maze than their WT littermates (+/+) and treatment with CX516 at 40 mg/kg did not produce a significant effect (genotype-treatment interaction:  $F_{1,43} = 0.295$ , NS; treatment effect:  $F_{1,21} = 2.108$ , NS; genotype effect:  $F_{1,21} = 28.314$ ,  $p < 0.001$ ). (B) *Scn2a*<sup>KO/+</sup> mice spent significantly more time in the open arms and this was not affected by CX516 injection (genotype-treatment interaction:  $F_{1,43} = 0.737$ , NS; treatment effect:  $F_{1,21} = 0.924$ , NS; genotype effect:  $F_{1,21} = 43.933$ ,  $p < 0.001$ ). (C) The number of entries in the open arms of the track was also increased in the *Scn2a*<sup>KO/+</sup> group and CX516 did not affect significantly this phenotype (genotype-treatment interaction:  $F_{1,43} = 0.001$ , NS; treatment effect:  $F_{1,21} = 0.122$ , NS; genotype effect:  $F_{1,21} = 58.400$ ,  $p < 0.001$ ). N = 11 for each group of genotype and each condition. Values are expressed as mean  $\pm$  standard error of the mean. Statistical significance was assessed using two-way ANOVA for genotype and treatment effect with significance set at (\*)  $p < 0.05$  and (\*\*\*)  $p < 0.001$ .

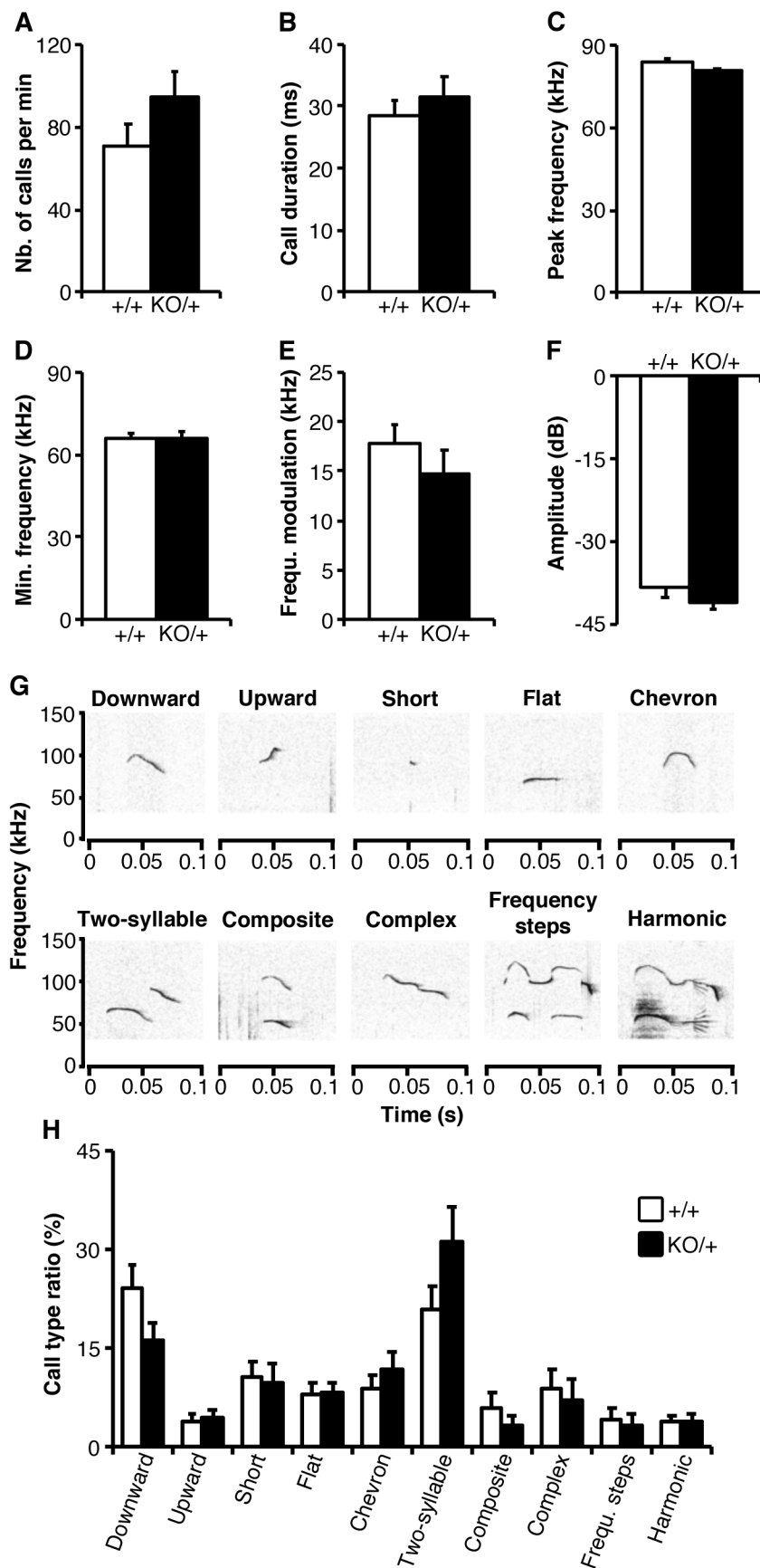


**Figure S6. CX516 injection does not affect significantly locomotor coordination performance.** Injection of CX516 in WT mice did not affect significantly the performance in the rotarod task at the different doses tested (trial-drug dose interaction:  $F_{15,80} = 0.899$ , *NS*; trial effect:  $F_{5,80} = 10.690$ ,  $p < 0.001$ ; drug dose effect:  $F_{3,16} = 0.260$ , *NS*).  $N = 5$  per condition. Values are expressed as mean  $\pm$  standard error of the mean. Statistical significance was assessed using two-way repeated measures ANOVA with significance set at  $p < 0.05$ .

### 3 CHAMBERS - HABITUATION



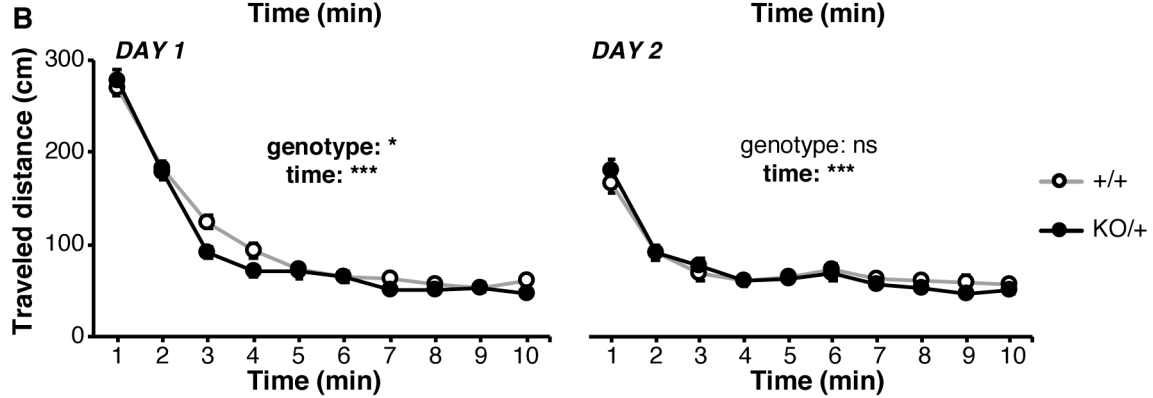
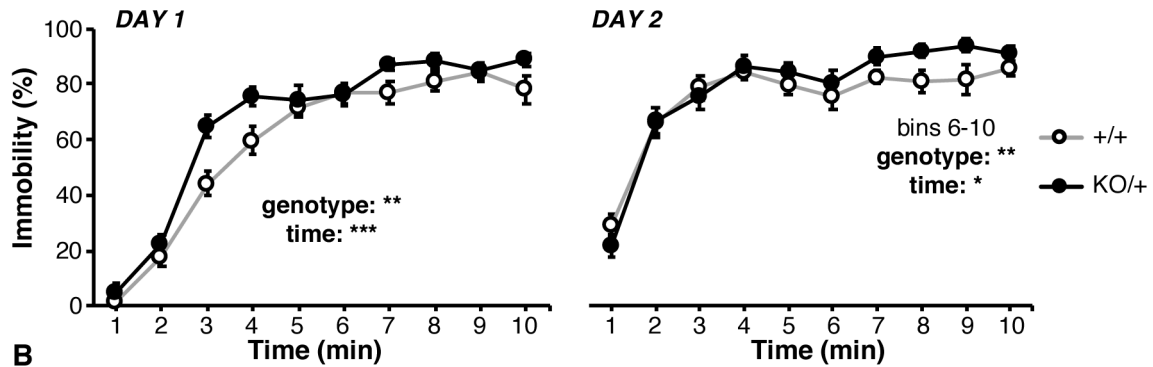
**Figure S7. Habituation and activity in the 3-chambers task were comparable between *Scn2a*<sup>KO/+</sup> and WT mice.** During the habituation to the maze, we did not observe a significant preference for a given side in either group. E1: Empty side 1; E2: Empty side 2. WT: N = 23, KO: N = 29. Values are expressed as mean  $\pm$  standard error of the mean. Statistical significance was assessed using Student t-test with significance set at  $p < 0.05$ .



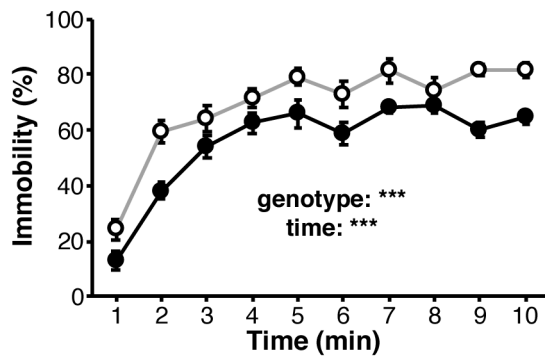
**Figure S8. Isolation-induced ultrasonic vocalizations are conserved in *Scn2a*<sup>KO/+</sup> pups at P6.** (A-F) Isolation-induced ultrasonic vocalizations recorded at postnatal 6 days were analyzed quantitatively and qualitatively using frequency, duration and amplitude parameters. *Scn2a*<sup>KO/+</sup> mice produced a number of calls (A) comparable to their WT littermates. The mean duration (B), peak frequency (C), minimum frequency (D), frequency modulation (E) and amplitude (F) were not significantly different between *Scn2a*<sup>KO/+</sup> and WT pups. (G) Calls were classified into the 10 main categories commonly observed in C57BL/6J pups. (H) The proportion of calls per category did not differ significantly between the two groups, even for downward and two-syllable calls that seemed decreased and increased respectively without reaching the significance level ( $p = 0.110$  and  $p = 0.122$  respectively). WT: N = 8, KO: N = 8. Values are expressed as mean  $\pm$  standard error of the mean. Statistical significance was assessed using Student t-test with significance set at  $p < 0.05$ .



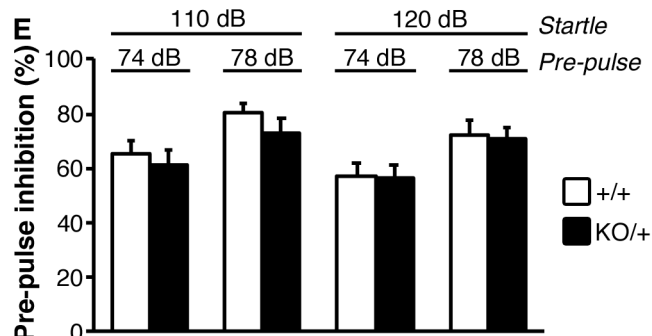
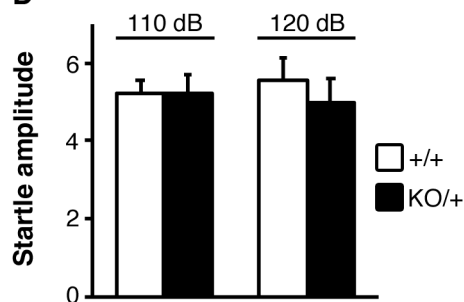
## A PORSOLT FORCED SWIM



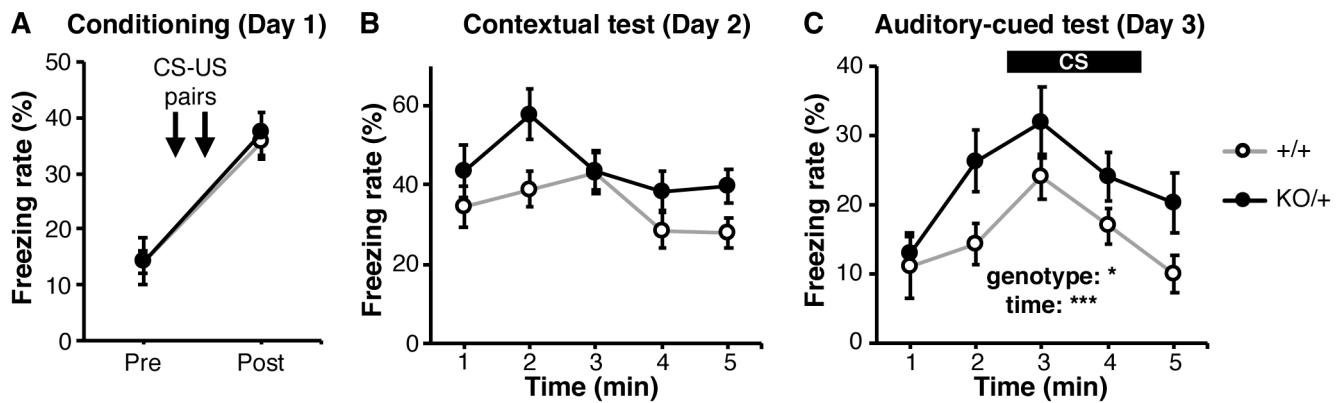
## C TAIL SUSPENSION TEST



## D PRE-PULSE INHIBITION

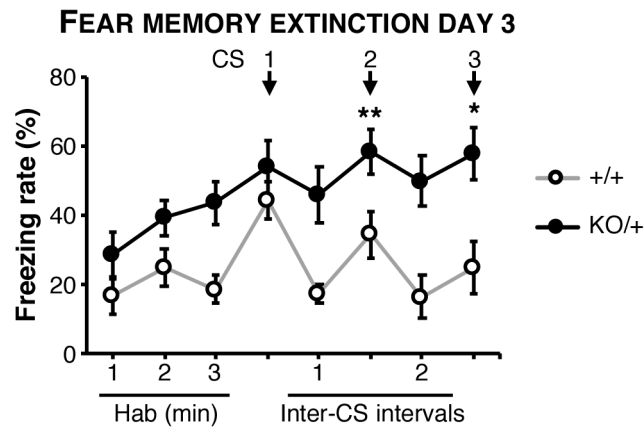


**Figure S9. *Scn2a*<sup>KO/+</sup> mice display despair behavior in the forced-swim task but conserved startle and pre-pulse inhibition response.** (A) In the Porsolt forced swim test, *Scn2a*<sup>KO/+</sup> mice were significantly more immobile than their WT littermates on day 1 and in the second half of day 2 (Day1: genotype-time interaction:  $F_{9,171} = 2.094$ , NS, time effect:  $F_{9,171} = 132.7$ ,  $p < 0.001$ ; genotype effect:  $F_{1,19} = 13.06$ ,  $p < 0.01$ ; Day2: genotype-time interaction:  $F_{9,171} = 1.661$ , NS, time effect:  $F_{9,171} = 48.420$ ,  $p < 0.001$ ; genotype effect:  $F_{1,19} = 3.102$ ,  $p > 0.01$ ; Day2: bins 6-10: genotype-time interaction:  $F_{4,76} = 0.533$ , NS, time effect:  $F_{4,76} = 3.077$ ,  $p < 0.05$ ; genotype effect:  $F_{1,19} = 8.572$ ,  $p < 0.01$ ). (B) Conversely, *Scn2a*<sup>KO/+</sup> mice traveled shorter distances on the first day tested, but not on the second day (Day 1: genotype-time interaction:  $F_{9,171} = 1.708$ , NS, time effect:  $F_{9,171} = 234.4$ ,  $p < 0.001$ ; genotype effect:  $F_{1,19} = 4.625$ ,  $p < 0.05$ ; Day 2: genotype-time interaction:  $F_{9,171} = 0.957$ , NS, time effect:  $F_{9,171} = 58.160$ ,  $p < 0.001$ ; genotype effect:  $F_{1,19} = 0.025$ , NS). (C) In the tail suspension test, *Scn2a*<sup>KO/+</sup> mice were significantly less immobile than their WT littermates (genotype-time interaction:  $F_{9,171} = 0.740$ , NS, time effect:  $F_{9,171} = 42.560$ ,  $p < 0.001$ ; genotype effect:  $F_{1,19} = 28.290$ ,  $p < 0.001$ ). (D) No significant differences were observed between the *Scn2a*<sup>KO/+</sup> and WT groups in startle response for the two sound levels used (110 dB and 120 dB). (E) The response to pre-pulse inhibition was also comparable between both groups for all the stimulation thresholds tested. WT: N = 20, KO: N = 20. Values are expressed as mean  $\pm$  standard error of the mean. Statistical significance was assessed using two-way repeated measures ANOVA (A-C) or two-way ANOVA (D,E) with significance set at  $p < 0.05$ .



**Figure S10. Freezing behavior is increased in *Scn2a*<sup>KO/+</sup> mice three days post-conditioning.**

Mice were tested in a fear conditioning, contextual and auditory cued testing paradigm. **(A)** In the conditioning phase of the fear conditioning test, the freezing behavior was significantly increased by two pairs of conditioned (CS, tone) and unconditioned stimuli (US, foot shock) in *Scn2a*<sup>KO/+</sup> and WT mice without significant genotype effect (genotype-time interaction:  $F_{1,12} = 0.075$ , *NS*; time effect:  $F_{1,12} = 48.570$ ,  $p < 0.001$ ; genotype effect:  $F_{1,12} = 0.039$ , *NS*). **(B)** After 24 hours, when mice were placed back in the same environment without CS or US, *Scn2a*<sup>KO/+</sup> mice tended to freeze more than their WT littermates even though this did not reach the significance level (genotype-time interaction:  $F_{4,48} = 1.644$ , *NS*; time effect:  $F_{4,48} = 48.570$ ,  $p < 0.01$ ; genotype effect:  $F_{1,12} = 4.574$ ,  $p = 0.086$ ). **(C)** In the auditory cued test, 24 hours later, mice were placed in a completely different environment and exposed to one CS without US. The freezing percentage was significantly higher in the *Scn2a*<sup>KO/+</sup> group (genotype-time interaction:  $F_{4,48} = 0.639$ , *NS*; time effect:  $F_{4,48} = 9.743$ ,  $p < 0.001$ ; genotype effect:  $F_{1,12} = 5.399$ ,  $p < 0.05$ ). WT: N = 13, KO: N = 13. Values are expressed as mean  $\pm$  standard error of the mean. Statistical significance was assessed using two-way repeated measures ANOVA with significance set at (\*)  $p < 0.05$ .



**Figure S11. Fear memory induced freezing remains abnormally elevated in *Scn2a*<sup>KO/+</sup> mice 48 hours after conditioning.** Mice were trained for fear conditioning by exposition to tone (CS: conditioned stimulus) paired to electric foot-shocks (US: unconditioned stimulus). Forty-eight hours post-conditioning, *Scn2a*<sup>KO/+</sup> mice showed a significantly stronger freezing behavior when exposed to three consecutive pairs of CS without US. WT: N = 11, KO: N = 12. Values are expressed as mean  $\pm$  standard error of the mean. Statistical significance was assessed using two-way repeated measures ANOVA followed by Sidak's multiple comparison post-hoc test with significance set at (\*)  $p < 0.05$  and (\*\*)  $p < 0.01$ .